

A Modulus™ Microplate Fluorometer Method for DNA Quantitation Using Quant-iT™ DNA HS Assay



INTRODUCTION

Quant-iT™ Assay Kits from Invitrogen are highly sensitive and convenient for DNA, RNA, and protein quantitation in microplate format. The Quant-iT™ DNA High-Sensitivity Assay is highly selective for dsDNA over RNA and tolerates salts, solvents, detergents, protein, and other common contaminants. When used with the Modulus™ Microplate Fluorometer, this assay provides an easy, accurate, and sensitive method for quantitation of DNA in small-volume microplates (200 µL per well). When read in the Modulus™ Microplate Fluorometer in conjunction with the Blue Fluorescence Optical Kit, the signal is linear from 30 pg to 100 ng DNA in 200 µL final volume (Figures 1 and 2).

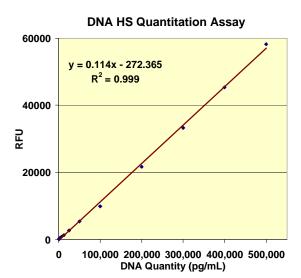
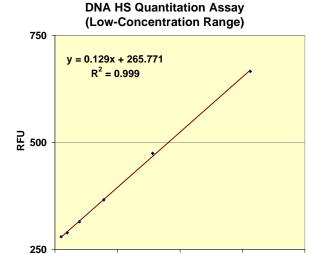


Figure 1. Serial dilutions of Quant-iTTM DNA HS samples analyzed using the ModulusTM Microplate Fluorometer and the Blue Fluorescence Optical Kit. 200 μL sample per well.



2,000

DNA Quantity (pg/mL)

3,000

4,000

Figure 2. Samples at low-concentration range analyzed using the Modulus™ Microplate Fluorometer and the Blue Fluorescence Optical Kit.

1,000

MATERIALS REQUIRED

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- □ Modulus[™] Microplate Fluorometer (P/N 9300-000) or Modulus[™] Microplate Multimode Reader (P/N 9300-002)
- □ Fluorescence Optical Kit Blue, 460/515-580 nm (P/N 9300-042)
- Quant-iT[™] DNA High-Sensitivity Assay Kit (Invitrogen, Q33120)
- □ 96-well, black microplates (Greiner Bio, FluoTrac 200, #655076)

EXPERIMENTAL PROTOCOL

1. Reagent Preparation Recommendation

Note: Handling, storage and use of the reagent should be performed in accordance with the product information sheet supplied by Invitrogen, Inc.

The Quant-iT[™] dsDNA HS Assay is supplied as a 1-mL concentrated dye solution in anhydrous dimethylsulfoxide (DMSO).



On the day of the experiment, equilibrate kit contents to room temperature.

Prepare a working solution by making a 1:200 dilution of the concentrated Quant-iT™ dsDNA HS reagent (Component A) in Quant-iT™ dsDNA HS buffer (Component B). Prepare this solution in a plastic container as the reagent may absorb into glass surfaces. Protect the working solution from light by covering it with foil or placing it in the dark.

Note: For best results, use this solution within three hours of preparation.

2. Instrument Set Up

- Insert the Blue Fluorescence Optical Kit (excitation 460 nm, emission 515-580 nm) into the Modulus™ Microplate according to the Operating Manual.
- From the Home screen, touch Select Protocol. Follow the protocol wizard to select the preset protocol Quant-iT[™] dsDNA High Sensitivity. Enter the following at the Select Protocol wizard: fluorescence; at the Preset tab, select Quant-iT[™] dsDNA High Sensitivity; Finish.
- The Instrument Control screen shows all the reading parameters: 1 sec integration, Blue Optical Kit, and all the wells in the plate are selected to read.
 Note: If necessary, use the Plate/Well selection icon to select or deselect the wells to be read. Green is selected and gray is deselected.
- Refer to the on-screen Help topics, Quick Start Guide, or Operating Manual for detailed instructions.

3. Sample Analysis

- Add 200 µL of sample to each well.
 Note: If necessary, use the Plate/Well selection icon to select or deselect the wells to be read. Green is selected and gray is deselected.
- Open the instrument door by using the Door icon on the touch screen. Place the plate with A1 well at the top right corner of the microplate sample tray. Close the door by using the Door icon.

- Touch the Start icon on the touch screen to begin reading.
- The Results screen displays the RFU value immediately after each well is measured.
- Once all measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive
- Remove the plate after measurement.

RESULTS

Sensitivity: typically 150 pg/mL

Dynamic Range: linearity range from 150 pg/mL to 500 ng/mL

Minimum Detection Limit*: 150 pg/mL, based on 3 STDV of blank controls, n = 24.

* data determined separately and not shown in this application note.

CONCLUSION

The Modulus Microplate™ Fluorometer offers superior sensitivity and dynamic range for detection of common fluorophors used in nucleic acid quantitation such as PicoGreen, RiboGreen, Alexa, and CyDyes. The Modulus™ Microplate Fluorometer achieves superior performance by using a dedicated fluorescence detector instead of sharing the detector with other detection modes. The individual optical kit with solid-state optics and a powerful wavelength-matched LED delivers excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

The superior performance, ease of use, and utmost flexibility of the Modulus™ Microplate make it an ideal microplate reader for today's life science laboratory.



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